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# Sea buckthorn in endodontics: A comprehensive review of Its antimicrobial efficacy against *Enterococcus faecalis* in root canal infections

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## Abstract

**AIM.** *Enterococcus faecalis* is a gram-positive facultative anaerobe commonly known as the primary pathogen implicated in persistent and secondary endodontic infections, due to its exceptional capacity to invade dentinal tubules, form resistant biofilms and survive in nutritionally adverse alkaline environments. Although effective, conventional chemical irrigants like sodium hypochlorite and chlorhexidine have been found to cause cytotoxicity and other unwanted effects. Sea buckthorn (*Hippophae rhamnoides* L.) is a medicinal plant that has shown promise as a natural antimicrobial agent due to its richness in flavonoids, polyphenols, carotenoids and unsaturated fatty acids. The purpose of this review is to summarize and examine existing evidence on the antimicrobial efficacy, mechanisms of action, and clinical potential of sea buckthorn against *E. faecalis* in the context of root canal infections.

**MATERIALS AND METHODS.** The literature review was conducted thoroughly by using the PubMed, Embase and Cochrane Library databases. The review incorporated studies in English that involved phytochemical, microbiological, in vitro, and in vivo studies that were relevant to the study involving the use of sea buckthorn-derived agents when conducting dentistry and endodontics research.

**RESULTS.** Extracts of sea buckthorn exhibit broad-spectrum antimicrobial activity that can be ascribed to bioactive compounds, such as quercetin, kaempferol, isorhamnetin, palmitoleic acid and ascorbic acid. Disruption of bacterial cell membranes, inhibition of biofilm formation, suppression of acid production, and modulation of host inflammatory responses are all reported antibacterial mechanisms. Aqueous and hydroalcoholic leaf extracts have demonstrated growth-inhibitory action against *E. faecalis*, whilst, pulp oil-based preparations have demonstrated anti-biofilm activity against a variety of oral pathogens. Comparative studies indicate similar, and in certain cases better, efficacy compared with chlorhexidine, and much better biocompatibility.

**CONCLUSIONS.** Sea buckthorn is a biocompatible, multifunctional adjunct that can be used as an endodontic irrigant or intracanal medicament. Nonetheless, before it can be adopted into routine clinical practice, standardization of extraction techniques, optimal concentrations, and optimally designed randomized clinical trials are required.

**Keywords:** sea buckthorn, *Hippophae rhamnoides*, *Enterococcus faecalis*, endodontics, root canal irrigants, herbal antimicrobials, biofilm



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# Облепиха в эндодонтии: систематический обзор антимикробной активности в отношении *Enterococcus faecalis* при инфекциях системы корневых каналов зубов

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## Резюме

**ЦЕЛЬ.** *Enterococcus faecalis* – грамположительная факультативно-анаэробная бактерия, широко признанная основным возбудителем персистирующих и вторичных эндодонтических инфекций благодаря своей исключительной способности проникать в дентинные каналы, формировать устойчивые биопленки и выживать в неблагоприятных щелочных условиях с ограниченным количеством питательных веществ. Несмотря на высокую эффективность традиционных химических ирригантов, таких как гипохлорит натрия и хлоргексидин, их применение связано с цитотоксичностью и рядом нежелательных побочных эффектов. Облепиха (*Hipporhae rhamnoides* L.) представляет собой лекарственное растение, обладающее значительным потенциалом в качестве природного антимикробного средства благодаря высокому содержанию флавоноидов, полифенолов, каротиноидов и ненасыщенных жирных кислот. Целью данного обзора является обобщение и анализ существующих данных об антимикробной эффективности облепихи против *E. faecalis*, механизмах ее действия и клиническом потенциале в лечении инфекций корневых каналов.

**МАТЕРИАЛЫ И МЕТОДЫ.** Поиск литературы был проведен в базах данных PubMed, Embase и Cochrane Library. В обзор были включены англоязычные публикации, посвященные фитохимическим, микробиологическим, *in vitro* и *in vivo* исследованиям, связанным с применением производных облепихи в стоматологии и эндодонтии.

**РЕЗУЛЬТАТЫ.** Экстракты облепихи демонстрируют широкий спектр антимикробной активности, обусловленной присутствием биологически активных соединений, таких как кверцетин, кемпферол, изорамнетин, пальмитолеиновая кислота и аскорбиновая кислота. К основным механизмам антибактериального действия относятся нарушение целостности клеточных мембран бактерий, ингибирование формирования биопленок, подавление продукции кислот и модуляция воспалительного ответа организма хозяина. Водные и водно-спиртовые экстракты листьев облепихи продемонстрировали способность подавлять рост *E. faecalis*, тогда как препараты на основе масла мякоти плодов проявляли выраженную антибиопленочную активность в отношении различных оральных патогенов. Сравнительные исследования свидетельствуют о сопоставимой, а в ряде случаев и более высокой эффективности по сравнению с хлоргексидином, при значительно лучшей биосовместимости.

**ВЫВОДЫ.** Облепиха является биосовместимым многофункциональным средством, которое может рассматриваться в качестве дополнительного эндодонтического ирриганта или внутриканального лекарственного препарата. Однако для ее внедрения в повседневную клиническую практику необходимы стандартизация методов экстракции, определение оптимальных концентраций и проведение качественно спланированных рандомизированных клинических исследований.

**Ключевые слова:** облепиха, *Hipporhae rhamnoides*, *Enterococcus faecalis*, эндодонтическое лечение, ирригация корневых каналов, фитопрепараты с антимикробным действием, бактериальные биопленки

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## INTRODUCTION

The objective of endodontic treatment is to remove microbial infection of the root canal system, and to prevent reinfection, thus preserving the natural dentition. The survival of microorganisms in the complex anatomy of root canals has been the main cause of failure in endodontic treatments [1]. *Enterococcus faecalis* has become the most commonly recovered and most clinically useful pathogen [2]. This gram-positive, facultative anaerobe contributes to the majority of persistent endodontic infections and has an unusual capacity to survive in the nutritionally depleted, alkaline environment of obturated root canals [3].

*E. faecalis* has various virulence factors that contribute to its virulence and persistence, such as biofilm formation, the ability to invade dentinal tubules, surface adhesins expression, gelatinase production, and hemolysin production, and resistance to multiple intracanal medicaments [4]. Research has shown that about 60–70% of clinical *E. faecalis* isolates have the capacity to form biofilms and that biofilm structure can lower effective concentration of the antimicrobial agents by orders of magnitude [5]. Its occurrence in failed root canal cases varies between 24 and 77% across different methods of detection and some molecular studies have also reported prevalence as high as 97.5% in refractory cases [6].

Traditionally, the root canal system is disinfected with sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX). Despite having a potent antimicrobial activity, these agents are associated with several limitations, such as cytotoxicity to periapical tissues, allergic reactions, undesirable taste, discoloration of dentin, and failure to completely eliminate biofilms within dentinal tubules [7–9]. Moreover, the unintentional leakage of NaOCl outside the apex may cause severe tissue damage. It is these limitations that have led to the pursuit of safer, biocompatible and equally effective alternatives, especially in the form of herbal or phytotherapeutic agents [10].

Sea buckthorn (*Hippophae rhamnoides* L.), a temperate shrub, is an evergreen, decadent, and widely spread shrub in the temperate regions of Asia and Europe, in addition to its long-standing use in traditional Tibetan, Mongolian, and Chinese medicine. The plant is remarkably rich in bioactive compounds (including flavonoids (quercetin, kaempferol, isorhamnetin), phenolic acids, carotenoids, vitamins (notably C, E, and K), unsaturated fatty acids (such as palmitoleic, oleic, and linoleic acids), and phytosterols [11; 12]. These constituents have a collective impact of providing antioxidant, anti-inflammatory, immunomodulatory, wound-healing, and broad-spectrum antimicrobial effects [13].

Recent studies have started to examine the potential of sea buckthorn-based preparations in dentistry, including their use in mouthwashes to promote periodontal health, in topical preparations to promote oral wound healing, and as adjunctive to the management of gingivitis and periodontitis [14; 15]. Nevertheless, the interest of sea buckthorn in endodontics, especially its role in the removal of *E. faecalis* in root canal infections,

is a relatively new field of research that has not been fully synthesized.

The aim of the review is to synthesize the available evidence on phytochemistry of sea buckthorn, its antimicrobial actions against endodontic pathogens, comparative effective action against *E. faecalis*, and possible clinical uses as either root canal irrigant or intracanal medicament. This paper will aim to identify research gaps and suggest directions of future research that can potentially translate the therapeutic potential of sea buckthorn into evidence-based endodontic practice.

## MATERIALS AND METHODS

### Search Strategy

An extensive literature search was done in electronic databases such as PubMed, Embase, and Cochrane Library. The search terms included were variations and combinations of “sea buckthorn”, “*Hippophae rhamnoides*”, “*Enterococcus faecalis*” and “endodontics” and “root canal irrigant” and “intracanal medicament” and “phytotherapy” and “herbal antimicrobial” and biofilm and dental caries. It was restricted to articles in the English language and performed microbial cultures, human subjects or other suitable *in vitro* models of the tooth. The search was carried out until the date of this review.

### Study Selection

The first search provided a considerable amount of articles. The inclusion criteria were studies focusing on the phytochemical composition, antimicrobial activity, biofilm-modulating activity, or clinical uses of sea buckthorn in dental or other microbiological related situations; studies that have investigated the pathogenesis, virulence or management of *E. faecalis* in endodontic infections; and studies that have evaluated the herbal or natural alternatives to conventional endodontic irrigants. The titles and abstracts of identified articles were screened by two independent reviewers, then the full-text review of the selected studies was undertaken. The disagreements were solved either by discussion or by the opinion of a third reviewer.

### Data Mining and Analysis

A standardized form was used to extract data of the selected studies. The extracted information included the characteristics of the studies, the type of preparation of sea buckthorn (extract, oil, juice, mouthwash), the target microbes, the study design (*in vitro*, *in vivo*, clinical), the comparator agents, the primary outcomes and the reported antimicrobial mechanisms. The heterogeneity of study designs, microbial models, and outcome measures led to the synthesis of the findings in a qualitative manner. Special consideration was given to publications that specifically study activity on *E. faecalis* or other pathogens of the oral cavity that have an interest in endodontic infections.

### Quality Assessment

The quality of the methodology of the chosen studies was determined to ascertain the reliability and validity of the research results presented in this review.

Two authors independently reviewed each of the studies, and any disagreements were discussed among the authors. The research was classified as low, moderate or high quality on the basis of the suitability of the methodology, sample size, controls and reproducibility. The emphasis in evidence synthesis was placed on high-quality studies. Following the inclusion and exclusion criteria, 28 articles were taken into consideration regarding the detailed review and inclusion in the given synthesis.

## RESULTS

### Endodontic Infection of *Enterococcus faecalis*

#### *Prevalence and Clinical Importance*

The most commonly isolated organism in teeth with persistent or secondary apical periodontitis is *E. faecalis*. It is estimated to cause about 24–77 percent of the isolates in failed endodontic cases, and its prevalence is much greater in retreatment cases than in primary infections [1; 4]. About 80–90% of the enterococcal infections in the mouth cavity are caused by *E. faecalis*, an organism that exhibits an incredible adaptability and tolerance to the severity of the environment, such as the extreme pH ranges and a long period of starvation [3; 5].

#### *Virulence Factors and Resistance Mechanisms*

The continued presence of *E. faecalis* in treated root canals is explained by the presence of several virulence factors. These adhesins, such as aggregation substance and enterococcal surface protein (Esp), mediate attachment to dentin and host tissues. Gelatinase and hemolysins production enables tissue destruction, whereas the formation of biofilms confer protection against antimicrobial agents and host immune responses [4; 6]. Notably, *E. faecalis* is able to enter dentinal tubules up to a depth of up to 1000  $\mu\text{m}$ , which is inaccessible to the conventional irrigation protocols. It is also able to survive in the alkaline environment formed by calcium hydroxide and has intrinsic resistance to a number of intracanal medicaments [7].

#### *Shortcoming of Traditional Disinfection*

NaOCl and CHX may be the gold standards in endodontic disinfection; however, neither of the two agents can completely eliminate *E. faecalis* biofilms in dentinal tubules. It has been demonstrated that NaOCl in concentrations less than 3% can even cause extracellular DNA-dependent biofilm formation, which is paradoxical [8]. Moreover, both of the agents are linked to cytotoxicity, possible allergic response, and the inability to infiltrate deeper layers of infected dentin. The limitations have led to the development of interest in herbal and natural alternative that is both antimicrobial and biocompatible [10].

#### *Sea Buckthorn Phytochemistry*

The berries of the sea buckthorn, as well as its seeds, leaves and bark, contain a complex of bioactive compounds, which have contributed to its pharmacological activity. The major classes of constituents and their reported activities are summarized in Table 1. The most common type of antimicrobial fraction is the flavonoids and phenolic acids, which are mostly abundant in the leaves [11]. Seed oil is also extremely rich in unsaturated fatty acids and tocopherols, as do berries [12]. The pulp oil is special in that it has a high content of palmitoleic acid (omega-7), an unsaturated fatty acid with reported antimicrobial activity against gram-positive bacteria [14].

Significantly, antimicrobial potency of sea buckthorn depends on part of the plant, solvent used in extraction process, and the cultivar. Comparative phytochemical analyses reveal that extracts of leaves will typically contain relatively higher levels of polyphenols and exhibit greater antibacterial activity than extracts of berries, whereas water and ethyl acetate fractions of seed and root extracts will demonstrate the strongest activity in some studies [11; 13]. This compositional heterogeneity provides a foundation to customize sea buckthorn-based preparations to specific clinical uses, such as, endodontic disinfection.

**Table 1.** Major phytochemical classes in *Hippophae rhamnoides* relevant to antimicrobial activity

**Таблица 1.** Основные фитохимические классы *Hippophae rhamnoides*, имеющие отношение к антимикробной активности

Class of compound	Representative constituents and reported bioactivity
Flavonoids	Quercetin, kaempferol, isorhamnetin, and their glycosides; antimicrobial, antioxidant, anti-biofilm, and anti-inflammatory activity
Phenolic acids	Ferulic, caffeic, gallic, and chlorogenic acids; disrupt bacterial cell membranes and inhibit microbial enzymes
Carotenoids	$\beta$ -carotene, lycopene, zeaxanthin, lutein; antioxidant and immunomodulatory activity
Vitamins	Vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin K; antioxidant, support tissue healing, and modulate microbial growth
Unsaturated fatty acids	Palmitoleic acid (omega-7), oleic acid, linoleic acid, $\alpha$ -linolenic acid; membrane-disruptive and anti-inflammatory effects
Phytosterols and triterpenoids	$\beta$ -sitosterol, ursolic and oleanolic acids; antimicrobial and tissue-protective effects
Tannins and proanthocyanidins	Inhibit microbial adhesion, reduce biofilm formation, and provide astringent action

**Table 2.** Proposed antimicrobial mechanisms of sea buckthorn relevant to *E. faecalis***Таблица 2.** Предложенные антимикробные механизмы облепихи, относящиеся к *E. faecalis*

Mechanism	Description and relevance to <i>E. faecalis</i>
Cell membrane disruption	Flavonoids and unsaturated fatty acids interact with bacterial phospholipid bilayers, increasing membrane permeability and leading to leakage of cellular contents
Inhibition of biofilm formation	Polyphenolic compounds interfere with quorum sensing and adhesion molecules, reducing the ability of <i>E. faecalis</i> to establish protective biofilms within dentinal tubules
Inhibition of microbial enzymes	Phenolic acids inhibit bacterial enzymes such as gelatinase and proteases that contribute to <i>E. faecalis</i> virulence and tissue invasion
Acid neutralization and antioxidant action	Vitamin C, carotenoids, and tocopherols neutralize reactive oxygen species, protect host tissues, and create an environment less favorable for acidogenic survival
Modulation of host immune response	Anti-inflammatory and immunomodulatory compounds enhance local host defenses against persistent endodontic pathogens
Synergy with conventional irrigants	Combination with low-concentration NaOCl or CHX may produce additive effects, potentially reducing required doses and associated cytotoxicity

### Antimicrobial Mechanisms Relevant to *E. faecalis*

The antimicrobial activity of sea buckthorn against oral and endodontic pathogens results from multiple, complementary mechanisms (Table 2). Flavonoids like quercetin and isorhamnetin interfere with the cytoplasmic membranes of bacteria, resulting in the leakage of the intracellular components and bacterial death. In addition to bacteriostatic and bactericidal effects, these compounds also inhibit DNA gyrase and other essential enzymes [15; 16]. Phenolic acids, especially ferulic and gallic acids, cause microbial proteins to denature and interfere with the biosynthesis of cell wall, whereas tannins and proanthocyanidins block the adhesion of microbes and prevent the initiation of biofilms [17].

The gram-positive bacteria, such as streptococci and *E. faecalis*, are directly membrane-disruptive by unsaturated fatty acids in sea buckthorn pulp oil, especially palmitoleic acid [14; 18]. The moderate acidity of the local pH due to a high concentration of vitamin C may inhibit acidogenic survival without incurring the irritant effects of strong synthetic acids. Also, the carotenoids and tocopherols neutralize the reactive oxygen species produced during inflammation and indirectly aid in the recovery of host tissues within and around the periapical area [19].

In the particular example of *E. faecalis*, polyphenolic fractions of sea buckthorn have been reported to inhibit the development of biofilms by disrupting the expression of adhesins and the quorum sensing pathways. This applies especially in endodontics where biofilm-mediated resistance is a key challenge in successful therapy [20; 21]. Although still under research, its synergistic effects with conventional irrigants suggest that the sea buckthorn extracts could potentially be used with low-dose NaOCl or CHX to enhance overall efficacy but decrease chemical cytotoxicity [22].

### Evidence on Antimicrobial Efficacy

#### *In Vitro Studies on E. faecalis and Related Organisms*

Direct *in vitro* data to support the activity of sea buckthorn against *E. faecalis* is gradually becoming available. Sea buckthorn leaves have been shown to have growth-inhibitory properties against the *E. faecalis*

organism, as well as other clinically significant organisms, including *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* [15]. Total phenolic and flavonoid content had a positive correlation with the antibacterial activity, which further validates the use of these compounds as the primary antimicrobial agents.

Extensive phytochemical and microbiological studies on the sea buckthorn berries, leaves, seeds, and roots have established that crude ethanolic extracts and their fraction are active against gram-positive and gram-negative microorganisms. The water and ethyl acetate fractions are likely to be more active compared to hexane fractions as the polar polyphenolic compounds are mostly dominant in the bioactive pool [13; 23; 24].

#### *Sea Buckthorn-Based Oral Care Formulations*

A groundbreaking study, assessing an oil-based mouthwash, derived from sea buckthorn pulp with active bactericidal and bacteriostatic properties against *Streptococcus gordonii* and *Porphyromonas gingivalis* respectively, and bactericidal effects with respect to *Actinomyces viscosus* [14; 25]. This anti-biofilm potency shown with *E. faecalis* was not the primary target organism of that study, but the demonstrated anti-biofilm potency is directly applicable to endodontic applications, considering the dominant role of biofilm in *E. faecalis*-associated treatment failures.

Studies utilizing berry juice have found that sea buckthorn juice, at 20% concentration, completely inhibits *in vitro* growth of a variety of oral streptococci, such as *S. mutans*, *S. sanguinis* and *S. gordonii*, with the antibacterial effect due at least in part to the low juice pH (4.15.4) [26]. Even though low pH does not necessarily imply clinical applicability of unprocessed sea buckthorn preparations, this observation highlights the inherent antimicrobial potential of untreated sea buckthorn preparations.

#### *Clinical Evidence in Oral Health*

Even though there are no clinical trials specifically assessing the use of sea buckthorn in endodontics, periodontal applications can provide information. The use of subgingival delivery of sea buckthorn thixotropic

solution as an adjunct to scaling and root planing has been demonstrated to have a positive effect on clinical parameters and the reduction of levels of *Aggregatibacter actinomycetemcomitans* in patients with chronic periodontitis [27]. These results indicate the potential of using sea buckthorn-based delivery system in the confined oral space, which is also a characteristic similar to the needs of intracanal medicament placement in endodontic treatment.

### Comparative Perspective: Sea Buckthorn Versus Conventional and Herbal Alternatives

Sea buckthorn extracts have the significant benefit of biocompatibility as compared to NaOCl and CHX. Whereas traditional irrigants are cytotoxic to periapical tissues at clinically achievable concentrations, sea buckthorn-based products have demonstrated positive safety profiles when used orally in the mucosal area, and other wound-healing and anti-inflammatory effects [12; 14]. Nevertheless, the antimicrobial efficacy of NaOCl, especially, its tissue-dissolving effect, is, as of now, stronger than any reported herbal counterpart, including sea buckthorn.

Sea buckthorn holds a somewhat under-researched yet potentially promising role when compared to other herbal endodontic agents like propolis, triphala, neem, aloe vera and green tea [10; 28]. At the same time as a few other phytotherapeutic agents offer a comparable combination of polyphenols, omega-7 fatty acids, and antioxidant vitamins [12]. Such a multifunctional profile could be especially beneficial in endodontic applications where antimicrobial action is required in combination with promoting the healing of periapical tissues.

### Potential Clinical Applications in Endodontics

#### *As a Root Canal Irrigant*

It may be possible to develop sea buckthorn extracts into root canal irrigation solutions either as standalone agents in low-risk situations or as adjuncts to the more traditional irrigants. The most probable candidate formulations are the aqueous or hydroalcoholic

leaf extracts at standardized phenolic levels, as they have been demonstrated to be active against *E. faecalis* and with minimal cytotoxicity [15]. The combination of protocols involving sea buckthorn extract, followed with EDTA to cleanse the sample of any bacteria, could be discussed to preserve the maximum of disinfection, and the integrity of dentin [22; 29].

#### *As an Intracanal Medicament*

Their viscous nature, which makes them suitable as multi-session endodontic treatment placement as intracanal medicaments between visits, is due to their viscous nature. These applications would take advantage of the extended contact duration necessary to treat *E. faecalis* biofilms in dentinal tubules. Intracanal medicaments based on sea buckthorn may have the potential to work together with calcium hydroxide to expand its antimicrobial spectrum and to reduce the known limitations of calcium hydroxide against *E. faecalis* [14; 25].

#### *As an Adjunct in Regenerative Endodontics*

In addition to the traditional disinfection, the wound-healing, antioxidant, and anti-inflammatory effects of sea buckthorn are a good fit to the objectives of regenerative endodontic therapy. Its components could be helpful in the maintenance of stem cell viability, local inflammation regulation, and pulp tissue regeneration in carefully controlled doses [12; 30]. This is a promising but by far untapped direction to future research.

### Limitations and Challenges

Although the initial results are promising, some limitations should be taken into consideration. Most of the available evidence of sea buckthorn are found in non-endodontic environments and studies that specifically target *E. faecalis* in dentinal tubule model are limited. This has resulted in an inconsistent phytochemical profile due to variability in extraction systems, solvent systems, parts of the plant, and cultivars, making cross-study comparisons challenging [11; 13]. Moreover, therapeutic concentrations and standardized formulations in terms of endodontic use are yet to be established.

**Table 3.** Selected studies evaluating the antimicrobial activity of sea buckthorn against oral and related pathogens

**Таблица 3.** Отдельные исследования, оценивающие антимикробную активность облепихи в отношении патогенов полости рта и связанных с ними микроорганизмов

Sea buckthorn preparation	Microbial target	Comparator	Reported outcome
Aqueous and hydroalcoholic leaf extracts	<i>E. faecalis</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>P. aeruginosa</i>	Standard antibiotic discs	Significant growth inhibition; potent antioxidant activity
Pulp oil-based mouthwash	<i>S. gordonii</i> , <i>P. gingivalis</i> , <i>A. viscosus</i> , <i>C. albicans</i>	Chlorhexidine, herbal mouthwash	Bactericidal effect; complete inhibition of biofilm formation
Berry juice (20%)	<i>S. mutans</i> , <i>S. mitis</i> , <i>S. sanguinis</i> , <i>S. gordonii</i>	Saline control	Total bacterial inhibition at low pH (4.1–5.4)
Thixotropic gel (subgingival delivery)	<i>A. actinomycetemcomitans</i>	Scaling and root planing alone	Adjunctive use improved clinical and microbiological outcomes in chronic periodontitis
Berry, seed, root, and stem extracts	Gram-positive and gram-negative organisms	Synthetic antimicrobials	Broad-spectrum antibacterial activity; strongest in seed and root water fractions

Stability of bioactive compounds of sea buckthorn during storage and in the oral environment also should be further investigated as polyphenols are prone to oxidation and degradation. Moreover, the possible interaction between sea buckthorn extracts and dentin substrates, restorative materials, and bonding systems have not been properly characterized. And lastly, although initial cytotoxicity studies are positive, extensive biocompatibility analyses with human periapical and pulpal cell lines are required preceding clinical translation [12; 31–33].

### Future Directions and Research Recommendations

The translational potential of sea buckthorn in endodontics requires coordinated research efforts in several priority areas.

#### *Standardized In Vitro Evaluation Against E. faecalis*

The standardized *E. faecalis* biofilm models in extracted human teeth should be used in future studies to enable a direct comparison of the three herbal agents of NaOCl, CHX, and others. Preparation, concentration, contact time and outcome measures (reduction of colony-forming units, biofilm biomass and dentinal tubule penetration) that must be standardized in order to generate clinically meaningful information.

#### *Identification of Active Fractions and Synergistic Combinations*

Bioassay-guided fractionation can identify the specific compounds responsible for the antimicrobial activity of sea buckthorn against *E. faecalis*. Once identified, these compounds could be formulated into purified or semi-purified preparations to ensure consistent potency. Synergistic studies combining sea buckthorn extracts with low-dose NaOCl, EDTA, or chitosan-based delivery systems should also be pursued.

#### *Advanced Delivery Systems*

Nanotechnology-based delivery platforms, such as nanoemulsions, liposomes, and polymeric nanoparticles, could be used to increase the penetration of sea buckthorn bioactives into dentinal tubules and enhance their stability. Those systems would be capable of overcoming the existing constraints related to the hydrophobic nature of some of the sea buckthorn constituents and permit controlled, sustained release in the root canal.

#### *Clinical Safety and Biocompatibility Studies*

Extensive in vitro cytotoxicity experiments on human periodontal ligament fibroblasts, dental pulp stem cells and osteoblasts are required in order to establish safety thresholds. These researches must be succeeded by

in vivo assessment in animal endodontic models and, eventually, well-designed randomized controlled trials in human subjects with primary and secondary endodontic infections.

#### *Biocompatibility and Clinical Safety Studies*

Comprehensive in vitro cytotoxicity studies on human periodontal ligament fibroblasts, dental pulp stem cells, and osteoblasts are needed to establish safety thresholds. These studies should be followed by in vivo evaluation in animal endodontic models and, ultimately, well-designed randomized controlled trials in human patients with primary and secondary endodontic infections.

#### *Integration into Evidence-Based Clinical Protocols*

When efficacy and safety have been established, sea buckthorn-based agents deserve to be considered as a part of complete endodontic disinfection regimens, with a focus on long-term outcomes such as periapical healing, the rates of treatment success, and patient-reported satisfaction. The collaboration between endodontists, microbiologists, phytochemists, and material scientists will be critical to successful clinical translation.

## CONCLUSION

The present review summarised existing evidence on the antimicrobial effects of sea buckthorn (*Hippophae rhamnoides* L.) on *Enterococcus faecalis* and its possible role in endodontic infection prevention. *E. faecalis* continues to be the major pathogen linked with the persistent endodontic infections, and traditional disinfection plans are constrained by cytotoxicity and incomplete elimination of biofilm. Sea buckthorn has a very distinct and varied phytochemical profile which encompasses flavonoids, phenolic acids, carotenoids, vitamins and unsaturated fatty acids which together confer to it broad-spectrum antimicrobial, anti-biofilm, antioxidant and tissue-protective effects.

Existing in vitro and clinical data in oral health care, though still early in the particular context of endodontics, confirm the promising potential of the sea buckthorn-derived agents as biocompatible replacements or adjuncts of traditional irrigants and intracanal medicaments. Before sea buckthorn can be a regular part of endodontics, standardization of extracts, identification of active fractions, testing in dentinal tubule biofilm models, and rigorously designed clinical trials are all necessary. The continued research could result in sea buckthorn becoming a valuable part of future, evidence-based approaches to disinfection of endodontic areas.

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